ROLE OF EXTRACELLULAR SIGNALS ON CELLULAR DIFFERENTIATION

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by

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Dedicated to

My Parents

Mr. Kamalesh Sharma & Mrs. Meenu Sharma

CERTIFICATE

This is to certify that the thesis entitled **"Role of Extracellular signals on Cellular differentiation"** being submitted by **Ms. Aarushi Sharma** to **Indian Institute of Technology Delhi** for the award of the degree of **"DOCTOR OF PHILOSOPHY",** is a record of the authentic research work carried out by her under my supervision and guidance. She has fulfilled all the requirements for submission of this thesis, which to the best of my knowledge has reached the required standard.

The material contained in this thesis has not been submitted in part or full to any other University or Institute for the award of any other degree.

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ABSTRACT:

Understanding the role of extracellular signals in extremely important for the development of tissue engineered models. For the transplantation of the tissue engineered models, there is need develop growth free approaches. These in vitro models will help in eliminating the immune rejection during transplantation. Alternatively, they will help in development of the personalized regenerative medicine for the patients. Thus, the present study deals with understanding the role of extracellular signals in tissue engineered models.

In the first study, we developed silk fibroin protein and gelatin-based conjugated bioink, which showed localized presence and sustained release of calcium. This study generated unprecedented mechanistic insights on the role of fibroin-gelatin-CaCl2 bioink in modulating expression of several proteins which are known to play crucial role in bone regeneration as well as key signalling pathways such as β-catenin, BMP signalling pathway, Parathyroid hormone-dependent signalling pathway, Forkhead box O (FOXO) pathway, and Hippo pathways in hMSC-laden bioprinted constructs.

In second study, we developed hybrid liver-specific three-dimensional (3D) printed scaffolds using a solubilized native decellularized liver (DCL) matrix and silk fibroin (SF) and investigated their ability to support functional cultures of hepatic cells. In comparison to 2D controls, hepatic cells cultured on 3D SG−DCL revealed increased proliferation until 2 weeks and an upregulated expression of hepatocyte markers, including asialoglycoprotein receptor 1 (ASGR1). The Wnt pathway gene β-catenin was upregulated by more than 4-fold in 3D SG−DCL on day 3, while it showed a decline on day 7 as compared to 3D SG and also 2D controls. The expression of the epithelial cell adhesion molecule (EpCAM) was however lower in both 2D SG−DCL (2-fold) and 3D SG−DCL (2.5-fold) as compared to that in 2D controls. Immunofluorescence studies validated the protein expression of ASGR1 in 3D SG−DCL. Albumin (ALB) was not identified on SG scaffolds but prominently expressed in 3D SG−DCL constructs. In comparison to 2D SG, both ALB (1.8-fold) and urea (5-fold) were enhanced in cells cultured on 3D SG−DCL on day 7 of culture. Hence, the SG−DCL 3D printed scaffolds provide a conducive microenvironment for elevating differentiation and functions of hepatic cells possibly through an involvement of the Wnt/β-catenin signalling pathway.

In third study, investigated the role of calcium supplementation in controlling macrophage phenotypes in pro-inflammatory and pre-reparative states. The involvement of

oxidative defence and mitochondria in cellular plasticity and the sequential M0 to M1 and M1 to M2 transitions was observed after calcium supplementation. This study describe the molecular mechanism of reactive oxygen species signalling and drive the interconnected cellular plasticity of macrophages in the presence of calcium. Gene expression analysis, followed by immunostaining, revealed a relationship between MHC class II maturation and cellular plasticity. It helped to elucidate the role of controlled calcium supplementation under various conditions. These findings underscore the molecular mechanism of calcium-mediated immune induction and its favourable use in different biomaterials, which is of great significance for tissue regeneration.

In fourth study, we examined the effect of paracrine factors secreted from the direct interaction of M2 macrophages and the 3D OA microcartilage model in the presence and absence of interleukins (IL). M2 macrophages stimulated the expression of collagen-2, matrix metalloproteinase (MMP)-1, and MMP-13 3D OA microcartilage models, indicating cartilage regeneration. This indicates that factors produced by M2 macrophages induce chondrocytes with anti-inflammatory and extracellular matrix (ECM) regeneration and remodelling properties, irrespective of exogenous ILs. Interestingly, the 3D OA microcartilage model de-differentiated M2 macrophages into monocytes and M1 macrophages, indicating probable crosstalk between both cell types. Paracrine factors of M2 macrophages and chondrocytes also help in reversing the 3D OA microcartilage model to healthy articular cartilage by upregulating the production of collagen-2 and not into fibrous cartilage by downregulating the production of collagen-1.

सार

ऊतक इंजीनियर मॉडल के विकास के लिए अत्यंत महत्वपूर्ण में बाह्य संकेतों की भूमिका को समझना। ऊतक इंजीनियर मॉडल के प्रत्यारोपण के लिए, विकास मुक्त दृष्टिकोण विकसित करने की आवश्यकता है। ये इन विट्रो मॉडल प्रत्यारोपण के दौरान प्रतिरक्षा अस्वीकृति को खत्म करने में मदद करेंगे। वैकल्पिक रूप से, वे रोगियों के लिए व्यक्तिगत पुनर्योजी दवा के विकास में मदद करेंगे। इस प्रकार, वर्तमान अध्ययन ऊतक इंजीनियर मॉडल में बाह्य संकेतों की भूमिका को समझने से संबंधित है।

पहले अध्ययन में, हमने सिल्क फाइब्रोइन प्रोटीन और जिलेटिन-आधारित संयुग्मित बायोइंक विकसित किया, जो स्थानीय उपस्थिति और कैल्शियम की निरंतर रिहाई को दर्शाता है। इस अध्ययन ने कई प्रोटीनों की अभिव्यक्ति को संशोधित करने में फाइब्रोइन-जिलेटिन-CaCl2 बायोइंक की भूमिका पर अभूतपूर्व यंत्रवत अंतर्दृष्टि उत्पन्न की, जो हड्डी पुनर्जनन में महत्वपूर्ण भूमिका निभाने के साथ-साथ β-कैटेनिन, बीएमपी सिग्नलिंग मार्ग, पैराथाइरॉइड हार्मोन जैसे प्रमुख सिग्नलिंग मार्ग में महत्वपूर्ण भूमिका निभाने के लिए जाने जाते हैं। -डिपेंडेंट सिग्नलिंग पाथवे, फोर्कहेड बॉक्स ओ (फॉक्सो) पाथवे, और हिप्पो पाथवे एचएमएससी से लदी बायोप्रिंटेड कंस्ट्रक्शन में।

दूसरे अध्ययन में, हमने एक घुलनशील देशी डीसेलुलराइज्ड लीवर (डीसीएल) मैट्रिक्स और सिल्क फाइब्रोइन (एसएफ) का उपयोग करके हाइब्रिड लीवर-विशिष्ट त्रि-आयामी (3D) मुद्रित मचान विकसित किए और यकृत कोशिकाओं की कार्यात्मक संस्कृतियों का समर्थन करने की उनकी क्षमता की जांच की। 2D नियंत्रणों की तुलना में, 3D एसजी–डीसीएल पर सुसंस्कृत यकृत कोशिकाओं ने 2 सप्ताह तक बढ़े हुए प्रसार और एसिआलोग्लाइकोप्रोटीन रिसेप्टर 1 (एएसजीआर1) सहित हेपेटोसाइट मार्करों की एक अपंजीकृत अभिव्यक्ति का खुलासा किया। Wnt पाथवे जीन β-कैटेनिन को 3 दिन में 3D एसजी–डीसीएल में 4 गुना से अधिक अपग्रेड किया गया था, जबकि 3डी एसजी और 2डी नियंत्रणों की तुलना में 7 दिन में गिरावट देखी गई। एपिथेलियल सेल आसंजन अणु (EpCAM) की अभिव्यक्ति हालांकि 2D SG–DCL (2-गुना) और 3D SG–DCL (2.5-गुना) दोनों में 2D नियंत्रणों की तुलना में कम थी। इम्यूनोफ्लोरेसेंस अध्ययनों ने 3D SG–DCL में ASGR1 की प्रोटीन अभिव्यक्ति को मान्य किया। एल्ब्यूमिन (ALB) की पहचान SG मचानों पर नहीं की गई थी, लेकिन 3D SG–DCL निर्माणों में प्रमुखता से व्यक्त की गई थी। 2D SG की तुलना में, ALB (1.8-गुना) और यूरिया (5-गुना) दोनों को संस्कृति के 7 दिन 3D SG–DCL पर संवर्धित कोशिकाओं में बढ़ाया गया था। इसलिए, SG–DCL 3D प्रिंटेड मचान संभवतः वनत/β- कैटेनिन सिग्नलिंग मार्ग की भागीदारी के माध्यम से यकृत कोशिकाओं के विभेदन और कार्यों को बढ़ाने के लिए एक अनुकूल माइक्रोएन्वायरमेंट प्रदान करते हैं।

तीसरे अध्ययन में, प्रो-इंफ्लेमेटरी और प्री-रिपेरेटिव अवस्थाओं में मैक्रोफेज फेनोटाइप को नियंत्रित करने में कैल्शियम सप्लीमेंट की भूमिका की जांच की गई। कोशिकीय प्लास्टिसिटी में ऑक्सीडेटिव रक्षा और माइटोकॉन्ड्रिया की भागीदारी और क्रमिक M0 से M1 और M1 से M2 संक्रमण कैल्शियम पूरकता के बाद देखे गए। यह अध्ययन प्रतिक्रियाशील ऑक्सीजन प्रजातियों के आणविक तंत्र का वर्णन करता है और कैल्शियम की उपस्थिति में मैक्रोफेज की परस्पर सेलुलर प्लास्टिसिटी को संकेत देता है। जीन अभिव्यक्ति विश्लेषण, उसके बाद प्रतिरक्षण के बाद, एमएचसी वर्ग II की परिपक्वता और सेलुलर प्लास्टिसिटी के बीच एक संबंध का पता चला। इसने विभिन्न परिस्थितियों में नियंत्रित कैल्शियम पूरकता की भूमिका को स्पष्ट करने में मदद की। ये निष्कर्ष कैल्शियम-मध्यस्थता प्रतिरक्षा प्रेरण के आणविक तंत्र और विभिन्न बायोमैटिरियल्स में इसके अनुकूल उपयोग को रेखांकित करते हैं, जो ऊतक पुनर्जनन के लिए बहुत महत्व रखता है।

चौथे अध्ययन में, हमने इंटरल्यूकिन्स (IL) की उपस्थिति और अनुपस्थिति में M2 मैक्रोफेज और 3D OA माइक्रोकार्टिलेज मॉडल की सीधी बातचीत से स्रावित पैरासरीन कारकों के प्रभाव की जांच की। M2 मैक्रोफेज ने कोलेजन -2, मैट्रिक्स मेटालोप्रोटीनेज (MMP) -1, और MMP-13 3D OA माइक्रोकार्टिलेज मॉडल की अभिव्यक्ति को प्रेरित किया, जो उपास्थि पुनर्जनन का संकेत देता है। यह इंगित करता है कि एम 2 मैक्रोफेज द्वारा उत्पादित कारक बहिर्जात आईएल के बावजूद, विरोधी भड़काऊ और बाह्य मैट्रिक्स (ईसीएम) पुनर्जनन और रीमॉडेलिंग गुणों के साथ चोंड्रोसाइट्स को प्रेरित करते हैं। दिलचस्प बात यह है कि 3डी ओए माइक्रोकार्टिलेज मॉडल ने एम2 मैक्रोफेज को मोनोसाइट्स और एम1 मैक्रोफेज में डी-विभेदित किया है, जो दोनों प्रकार के सेल के बीच संभावित क्रॉसस्टॉक को दर्शाता है। M2 मैक्रोफेज और चोंड्रोसाइट्स के पैरासरीन कारक कोलेजन -2 के उत्पादन को बढ़ाकर स्वस्थ आर्टिकुलर कार्टिलेज में 3D OA माइक्रोकार्टिलेज मॉडल को उलटने में मदद करते हैं न कि कोलेजन -1 के उत्पादन को कम करके रेशेदार उपास्थि में।

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